

FORMATION OF β -LACTOSE FROM THE STABLE FORMS OF ANHYDROUS α -LACTOSE

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ABSTRACT

Treatment of either of the stable anhydrous crystalline forms of α -lactose with potassium methoxide in methanol at reflux does not result in the formation of β -lactose, whereas the latter is formed from α -lactose monohydrate or hygroscopic anhydrous α -lactose under these conditions. We find that when β -lactose is added to a reaction mixture containing either form of stable anhydrous α -lactose, conversion of α -lactose to β -lactose proceeds readily. Further, we show that this conversion may be a solid-solid transformation rather than dissolution of α -lactose followed by crystallization as β -lactose.

INTRODUCTION

THE USE OF α -lactose monohydrate in a wide variety of baking applications has been studied by Ash (1976) who reported one of the shortcomings of α -lactose monohydrate to be its low solubility in baking formulations. β -Lactose has greater solubility than α -lactose monohydrate (Whittier, 1944), and the formation of β -lactose from deionized whey ultrafiltrate (Kavanagh, 1975) and its application in cake-baking (Goldman and Short, 1977) indicate a renewed interest in this form of lactose. Formation of β -lactose from α -lactose monohydrate with sodium hydroxide (Olano and Rios, 1978) or potassium methoxide (Parrish et al., 1979a) has been reported, but under the same conditions stable anhydrous forms of α -lactose were unaffected (Olano, 1978; Parrish et al., 1979a). This difference in behavior between α -lactose monohydrate and stable anhydrous α -lactose was surprising to us and led us to study further how stable anhydrous α -lactose behaved in basic methanol. We are able now to transform stable anhydrous forms of α -lactose to β -lactose, and we report here on the conditions necessary for this change and its possible mechanism.

EXPERIMENTAL

Materials

α -Lactose monohydrate was obtained from Sigma Chemical Co., St. Louis, Mo. β -Lactose was prepared by the procedure of Buma and van der Veen (1974). Stable anhydrous α -lactose was formed from α -lactose monohydrate by treatment with methanol (α_M) (Tanret, 1896; Lim and Nickerson, 1973) or by heating in air at 130°C (α_S) (Sharp, 1943). An unstable form of anhydrous lactose (α_H), containing variable amounts of β -anomer, was made by heating α -lactose monohydrate in vacuum at 130°C (Herrington, 1948). Amorphous lactose containing the α - and β -anomers was obtained by freeze-drying an aqueous solution of α -lactose monohydrate which had been allowed to attain mutarotational equilibrium (Roetman and van Schaik, 1975). Compounds containing the anomeric forms of anhydrous lactose in the ratio $\alpha:\beta = 4:1$ (Olano et al., 1977) or $\alpha:\beta = 5:3$ (Hockett and Hudson, 1931) were prepared by treating α -lactose monohydrate with 2% hydrogen chloride in anhydrous methanol and 1% aqueous methanol, respectively.

Potassium methoxide was obtained from Ventron Corp., Danvers, Mass., and potassium hydroxide and methanol were AR grade chemicals from Fisher Chemical Co., King of Prussia, Pa.

Apparatus

Optical rotations were measured at 589 nm and 20°C with an automatic polarimeter Model 141 (Perkin-Elmer, Norwalk, Conn.). Gas-liquid chromatography of trimethylsilyl ether derivatives of α - and β -lactose, prepared by the procedure of Sweeley et al. (1963), was performed on a 4 ft \times 1/8 in. column of 3% SP-2100 on Supelcoport (100–200 mesh) with temperature programming from 150–210°C on a Model 5750 instrument (Hewlett-Packard, Palo Alto, Calif.). Other parameters were helium flow-rate 30 ml/min, injector temperature 215°C, and detector temperature 220°C. The output signal from the flame ionization detector was transferred to an IBM 1130 computing system to calculate peak areas. Moisture determinations were made with an Aquatest II instrument (Photovolt Corporation, New York, N.Y.), and differential scanning calorimetry (DSC) measurements with a Model 990 thermal analyzer (DuPont, Wilmington, Del.). A JEOL FX-60Q NMR spectrometer (JEOL, Inc., Cranford, N.J.) was used to measure methanol and ethanol retained by the lactose samples (Parrish et al., 1979b). Atomic absorption analyses for potassium were made with a Model 306 instrument (Perkin-Elmer, Norwalk, Conn.) after ashing the sample in an electric furnace Type 056-PT (Hevi Duty Electric Co., Milwaukee, Wis.).

General procedure

The lactose sample was stirred at room temperature or at reflux for the required time with eight times its weight of 0.014M potassium hydroxide or potassium methoxide in anhydrous methanol or 1% aqueous methanol. The solid material was removed by filtration and, after being thoroughly washed with methanol, was dried in vacuum at 65°C for 16 hr. Mixtures of different forms of lactose were treated in the same way.

In other experiments, two different forms of lactose (2.5g each) were treated at reflux with basic methanol (100 ml) in the same reaction vessel, but with the two forms physically separated from one another by placing one form inside a cellulose Soxhlet extraction thimble (22 \times 80 mm); isolation of products was performed as described above.

Anhydrous α -lactose α_M , or α_S that had been treated with methanol at reflux for 2 hr to incorporate methanol, was mixed with an equal weight of β -lactose. The mixture was stirred at reflux for 3 hr with eight times its weight of 0.014M potassium ethoxide in ethanol. The solid material was removed by filtration and, after being thoroughly washed with ethanol, was dried in vacuum at 65°C for 16 hr. The methanol and ethanol contents of the product were measured by a proton magnetic resonance difference spectra method (Parrish et al., 1979b).

RESULTS & DISCUSSION

WE REPORTED previously (Parrish et al., 1979a) that, while α -lactose monohydrate is converted readily to β -lactose by treatment with methanolic potassium methoxide, the stable anhydrous crystalline forms of α -lactose, α_S (Sharp, 1943), and α_M (Tanret, 1896; Lim and Nickerson, 1973) were unchanged by this treatment (Table 1). This unexpected result led us to consider the possibility that the presence of water (derived from the water of crystallization of the monohydrate) in the methanol was a critical requirement for transformation to β -lactose. However, treatment of either of the anhydrous forms of α -lactose with potassium methoxide in methanol containing up to 1.5% water failed to produce β -lactose, thereby eliminating from consideration a role for water in this transformation. In addition, we examined the reaction of the compounds containing the anomeric forms of anhydrous lactose in the ratio $\alpha:\beta = 4:1$ (Olano et al., 1977) and $\alpha:\beta = 5:3$ (Hockett and

Table 1—Anomeric composition^a of lactose samples before and after treatment with potassium methoxide solution at reflux for 3 hr

Sample	Treatment			
	None		Potassium methoxide	
	α -Lactose	β -Lactose	α -Lactose	β -Lactose
α -Lactose monohydrate	100	0	5.0	95.0
Anhydrous α -lactose, α_s	98.4	1.6	97.8	2.2
Anhydrous α -lactose, α_M	98.8	1.2	98.1	1.9
Compound $\alpha:\beta = 4:1$	81.6	18.4	3.4	96.6
Compound $\alpha:\beta = 5:3$	62.2	37.8	5.0	95.0
Amorphous lactose	55.5	44.5	4.5	95.5
Anhydrous lactose, α_H	91.5	8.5	4.5	95.5

^a Determined by gas-liquid chromatography of trimethylsilyl ether derivatives

Hudson, 1931) with potassium methoxide in anhydrous methanol (Parrish et al., 1979a) and found that both of these molecular compounds were converted to β -lactose in the absence of water.

Observing that amorphous lactose (containing 2.5% or 5.0% water) (Roetman and van Schaik, 1975) was converted to crystalline β -lactose on treatment with methanolic potassium methoxide (Table 1), we focused our attention on β -lactose addition to effect the transformation of the stable anhydrous forms of α -lactose to β -lactose. At reflux for 3 hr in 0.014M potassium methoxide in methanol, addition of crystalline β -lactose to either of the stable anhydrous forms of α -lactose led to the formation of an additional amount of β -lactose, the increase depending on the initial amounts of the two lactose anomers in the mixture (Table 2).

We then examined the effect of physical separation of either form of stable anhydrous α -lactose from β -lactose under the conditions of 3-hr reflux in 0.014M potassium methoxide in methanol. Separation of equal weights of the two forms of lactose was achieved by use of a cellulose Soxhlet extraction thimble (22 × 80 mm); each component was weighed accurately. Each form of lactose was recovered unchanged in anomeric composition in 86–93% yield. Identical results were obtained irrespective of which form of lactose was placed inside the Soxhlet thimble. Conversion to β -lactose occurred when either form of stable anhydrous α -lactose together with β -lactose was placed inside the Soxhlet thimble and treated with potassium methoxide solution; this showed that the effect of the Soxhlet thimble in preventing transformation of the separated compounds to β -lactose is entirely due to physical separation of the components.

Another form of anhydrous lactose (Herrington, 1948), which we have designated as α_H to indicate its hygroscopic nature, has been shown by optical rotation measurements to contain 5–10% β -lactose (Buma and Wieggers, 1967). We have found that the β -lactose content of α_H prepared on different occasions can vary from 1–25%, although samples prepared in triplicate at any given time were identical. In our initial experiments we were unable to convert α_H to β -lactose with potassium methoxide (Parrish et al., 1979a), and in experiments with alcoholic sodium hydroxide solution Olano (1978) was also unable to transform completely α_H to β -lactose. In our present work we found that when α_H was prepared and treated immediately with alcoholic

Table 2—Effect of amount of added β -lactose on transformation of α -lactose to β -lactose in methanolic potassium methoxide at reflux for 3 hr

% β -Lactose ^a	
Initial	Final
2.0	9.1
5.0	25.2
10.2	39.1
20.2	51.5
27.1	68.2
34.0	95.9
42.2	96.5
48.6	95.9

^a Determined by gas-liquid chromatography of trimethylsilyl ether derivatives.

potassium methoxide or sodium hydroxide, conversion to β -lactose was complete (Table 1). Possibly when α_H was not processed immediately it was converted during the observed period of rapid weight increase at ambient relative humidity to a form like α_s which is not convertible to β -lactose with potassium methoxide.

The conversion of α_M or α_s to β -lactose through the influence of added crystalline β -lactose can be effected by addition of α -lactose monohydrate or amorphous lactose instead of crystalline β -lactose. This is because both of these forms of lactose with potassium methoxide produced crystalline β -lactose. Again there was the requirement that the added α -lactose monohydrate or amorphous lactose comprise at least one-third or one-half, respectively, of the mixture with the stable anhydrous forms of α -lactose in order that conversion to crystalline β -lactose be completed under the specified reaction conditions.

These observations that mixtures of α -lactose monohydrate, amorphous lactose, or β -lactose with stable anhydrous forms of α -lactose were converted to β -lactose with potassium methoxide solution precluded the use of this method for analysis of mixtures of different forms of lactose, e.g., determination of α -lactose monohydrate in the presence of other crystalline forms. This analytical approach appeared possible from the previous results of the behavior of the individual forms of lactose (Parrish et al., 1979a) before the interaction effects described above were observed.

Attempted conversions of the stable anhydrous forms of α -lactose, α_M or α_s , to β -lactose by reaction with potassium methoxide solution at 27°C in the presence of β -lactose or α -lactose monohydrate were much slower than reactions at reflux. This is shown for reactions of α_M or α_s with different levels of added β -lactose for 16 hr at 27°C (Table 3). It

Table 3—Effect of addition of crystalline β -lactose to α_M or α_s on extent of β -lactose formation at 27°C in 0.014M potassium methoxide in methanol

Time (hr)	% β -Lactose ^a		
	Initial	Final	
		With α_M	With α_s
140	0.4	0.6	0.5
16	23.9	37.7	37.4
3	48.4	50.0	49.6
6	48.4	59.4	56.7
16	48.4	75.5	70.6
24	48.4	83.8	77.4
48	48.4	95.9	96.0
16	78.3	96.1	96.0

^a Determined by gas-liquid chromatography of trimethylsilyl ether derivatives

Table 4—Proton magnetic resonance analysis of retention of methanol after treatment of α_M or methanol-treated α_s with β -lactose in 0.014M potassium ethoxide in ethanol

Sample	Methanol content in wt %		Retention % of methanol
	Before treatment	After treatment	
α_M	0.79	0.78	99
α_s (methanol-treated)	0.14	0.14	100
β	0	0	—
$\alpha_M + \beta$	0.39	0.34	87
α_s (methanol-treated) + β	0.07	0.06	86

is of interest that α_H was converted to β -lactose at 27°C. Recovery of products from reactions for up to 24 hr at 27°C were 97–100% compared to 86–93% for reactions for 3 hr at reflux.

All products were examined by polarimetry (Buma and van der Veen, 1974) as well as by gas-liquid chromatography (Sweeley et al., 1963). This was done to compare the anomeric composition data from the two methods, the correlation coefficient being 0.99. Purity (as % total anhydrous lactose) of β -lactose samples prepared by potassium methoxide treatment was >99% found by use of accurate equilibrium optical rotation data of Buma and van der Veen (1974). No carbohydrates other than lactose were detected by gas-liquid chromatography in any of the solid products of potassium methoxide treatment of lactose samples, although small amounts of galactose, lactulose, and saccharinic acids in the presence of much larger quantities of lactose were found in the filtrates from the reaction mixtures. The extent of incorporation of potassium into the solid products did not exceed 0.001g atoms/mole lactose as determined by atomic absorption spectroscopy. All products after potassium methoxide treatment were shown to be anhydrous by automated Karl Fischer titration. The lactose samples did retain methanol (0.17–0.80%) as the only detected impurity, the level of impurity being measurable also by DSC (Parrish et al., 1979b).

Olano and Rios (1978) explained the formation of β -lactose from α -lactose monohydrate in methanolic sodium hydroxide solution in terms of a three step process: dissolution of α -lactose monohydrate, rapid mutarotation in solution of α -lactose to an equilibrium anomeric mixture at a rate faster than the rate of crystallization of anhydrous α -lactose, and crystallization of β -lactose after its concentration reaches the saturation value. This interpretation does not explain why α_M and α_s , which we find to have solubilities in methanolic sodium hydroxide or potassium methoxide comparable to that of α -lactose monohydrate, were not converted to β -lactose (Olano, 1978; Parrish et al., 1979a). In addition, the theory involving dissolution of starting materials followed by crystallization of β -lactose cannot explain the lack of formation of β -lactose when either α_M or α_s was separated from β -lactose during the reaction in a Soxhlet thimble. The extent of formation of β -lactose in mixtures of α_M or α_s with β -lactose under our reaction conditions depended on the amount of added β -lactose (Table 2).

These facts lead us to suggest the possibility that the transformation of α_M or α_s to β -lactose in the presence of crystalline β -lactose is a solid-solid reaction. The formation of β -lactose by treatment of amorphous lactose with potassium methoxide may involve the initial formation of a mixture of crystalline α - and β -lactose prior to complete conversion to β -lactose; this crystallization process would be

analogous to that observed when amorphous lactose was treated with methanol (Ross, 1978). The same explanation can be advanced for the conversion of the two different anomeric compounds of lactose (Hockett and Hudson, 1931; Olano et al., 1977) to β -lactose (Table 1). In addition, we suggest that the formation of β -lactose from α -lactose monohydrate or α_H by treatment with potassium methoxide is also a solid-solid transformation wherein the expanded crystal form of anhydrous α -lactose (Berlin et al., 1972) reverts to the dense crystalline β -lactose.

In support of the idea that the transformation of α_M and α_s in the presence of β -lactose occurs without dissolution was the following observation. The β -lactose produced from α_M or methanol-treated α_s by treatment with 0.014M potassium ethoxide in ethanol retained the methanol associated with the α -lactose component as judged by alcohol analysis by a proton magnetic resonance difference spectra method (Parrish et al., 1979b) (Table 4). If dissolution of the α -lactose component had preceded crystallization in the β -form, no methanol would have been found in the product. No ethanol was incorporated into the β -lactose product (Table 4).

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